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PRIMER NOTE

Microsatellite markers for the wasp *Euodynerus foraminatus* (Vespidae: Eumeninae)

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Abstract

We have identified five polymorphic microsatellite loci for the solitary wasp *Euodynerus foraminatus*, using a partial genomic library constructed from random amplified polymorphic DNA fragments. We detected between three and 13 alleles per locus in a sample of 30 female wasps collected in southwest Michigan, USA. Observed heterozygosities ranged from 0.2 to 0.73. These markers will be used in our ongoing studies of sex determination and naturally occurring inbreeding in this species.

Keywords: *Euodynerus foraminatus*, Eumeninae, microsatellites, Vespidae

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Euodynerus foraminatus is a solitary caterpillar-hunting wasp, common in temperate North America. Females nest in pre-existing cavities and are easily collected from trap nests (Krombein 1967). Field observations suggest that sib-mating is common in *E. foraminatus* (Cowan 1979), but breeding experiments in our laboratory suggest that this species has single-locus complementary sex determination (sl-CSD). Widespread in Hymenoptera (Butcher *et al*. 2000), sl-CSD is incompatible with inbreeding because among sibling matings, 25% of the fertilized eggs develop as diploid males (Cook 1993) which are generally infertile (el Agoze *et al*. 1994). Testing for sl-CSD requires co-dominant markers such as microsatellites to identify biparental diploids. We identified five variable microsatellite loci from *E. foraminatus* using a partial genomic library constructed from random amplified polymorphic DNA (RAPD) fragments (Ender *et al*. 1996).

DNA was isolated from the whole thorax and abdomen of two virgin *E. foraminatus* females using a Puregene kit (Gentra Systems). An aliquot of each stock extraction solution was diluted 100 : 1 in TE buffer (Ausubel *et al*. 1992) and amplified using 26 RAPD primers (Operon Technologies OPB-3, OPC-11, OPC-13, OPC-17, OPD-1 through 20, and University of British Columbia UBC-406 and UBC-414) in 50-μL reactions, each consisting of 37.15 μL water, 5 μL 10× *Taq* DNA Polymerase chain reaction (PCR) buffer, 2 μL 50 mM $MgCl_2$, 0.5 μL 10 mM dNTP mix, 2 μL 5 μM primer solution, 0.35 μL *Taq* polymerase, and 3 μL DNA solution (all PCR reagents from Life Technologies). Amplifications were performed using an MJ Research PTC-100 with an initial denaturation step at 94 °C for 1 min, followed by 40 cycles of 94 °C for 30 s, 35 °C for 1 min, and 72 °C for 2 min, and a final extension at 72 °C for 30 min. For each product producing bands on a 1.5% agarose gel, we prepared Southern blots (Southern 1975; Ausubel *et al*. 1992). Single-stranded 24-base DNA probes for 12 di- and trinucleotide sequences (Glenn 1996) were labelled using a Gene Images 3′ Oligolabelling and ECF Amplification System kit (Amersham Pharmacia) and hybridized to blots. Blots were viewed on a Molecular Dynamics Storm 860 fluorescent imager. RAPDs yielding positives were repeated, and the products were used to transform bacteria using an Invitrogen TOPO-TA cloning kit. We prepared colony lifts (Glenn 1996) and probed them as described above. Inserts from 50 positive colonies were amplified in cocktails of 40.8 μL water, 5 μL 10× PCR buffer, 1.5 μL 50 mM $MgCl_2$, 0.5 μL 10 mM dNTPs, 1 μL 25 μM T7 primer, 1 μL 25 μM M13 primer, and 0.18 μL *Taq* polymerase, with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 5 min. Twenty-four inserts with distinct sizes were sequenced with T7 and M13R primers, using the ABI PRISM BigDye Terminator kit and an ABI PRISM 310 genetic analyser. Sequences were screened for repeats, and to rule out overlaps, using ABI PRISM AUTOASSEMBLER 2.1. Four AT or GC repeat sequences, for which no probes were used, were also

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Table 1 Repeat motifs, primer and allele characteristics, and polymorphism data for five *Euodynerus foraminatus* microsatellite loci

Locus	GenBank accession number	Repeat motif	Primer sequences (5′–3′)	No. of alleles	Allele size range (bp)	*N*	H_O	H_E
Efo01	AF485776	$(CTT)_9$	F: GGAGAATCTGTCGAGTGTGAGAG R: GCTCTTTCCTTTTCTTACGAATATG	3	194–200	30	0.37	0.44
Efo02	AF485777	$(AT)_7$	F: GAATTTATGAAATTACGAATGAAACG R: GTTACCACGATGTATAGATATTAGG	6	200–210	30	0.60	0.57
Efo03	AF485778	$(GC)_5(GT)_{10}$	F: CATTTCAGAATAGTATATGAATGTG R: TTAATTTGTATATGCGTTGACACG	13	111–139	30	0.73	0.88
Efo04	AF485779	$(AG)_4N_4(AG)_3N_2(AG)_4$	F: ATAAGCGATCAAAGATAAGCGTC R: TTCTACTTGACAGAACTTGGCTC	12	211–245	30	0.70	0.89
Efo07	AF485782	$(AT)_5$	F: AAAATTCTGATCCAGATTCTATGG R: GACTAAACGAAAGCGATTAGAG	4	182–188	30	0.20	0.29

N, number of individuals, H_O, observed heterozygosity, H_E, expected heterozygosity.

found during sequencing. We designed primers for eight sequences, using OLIGO 6.3 (Molecular Biology Insights). We tested primers using touchdown PCR with an initial denaturing step of 94 °C for 1 min, followed by 10 cycles of 94 °C for 30 s, 60 °C for 30 s decremented by 1 °C per cycle, and 72 °C for 45 s, and an additional 25 cycles of 94 °C for 15 s, 50 °C for 15 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. Seven of the eight primer pairs yielded single products and were selected for fragment analysis.

We isolated DNA from one antenna of each of 30 females collected from separate nests in southwest Michigan and rehydrated each sample in 100 μL TE buffer. DNA was amplified in a 15-μL reaction consisting of 11.25 μL water, 1.5 μL 10× buffer, 0.75 μL 50 mM $MgCl_2$, 0.2 μL 10 mM dNTPs, 0.1 μL 20 μM forward primer labelled with amine-reactive BCI dye (ResGen), 0.1 μL 20 μM reverse primer, 0.1 μL Platinum *Taq* polymerase (Life Technologies), and 1 μL of DNA solution, using the above touchdown PCR protocol. Fragment analysis was performed on a Beckman Coulter CEQ 2000 XL DNA analyser.

Five of the seven loci were polymorphic in *E. foraminatus* (Table 1). Discrepancies between observed and expected heterozygosities varied among loci. Although we cannot draw firm conclusions about population structure from this sample, these markers will become important for studying ploidy levels and inbreeding in *E. foraminatus*.

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